STUDIES ON EXPERIMENTAL HAPLOIDY IN SALAMANDER LARVAE

II. Cytological Studies on Androgenetic Eggs of Triturus viridescens

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Introduction

One of the outstanding features of the experiments on androgenesis with eggs of salamanders has been the high rate of mortality during cleavage and gastrulation (Fankhauser, 1934*a*; Kaylor, 1937). However, there have been surprisingly few investigations on the cytology of failure of development in these early stages of androgenetic development. The most extensive observations have been those of Fankhauser (1934, *a* and *b*) on androgenetic egg fragments of *Triton palmatus* and more recently of Fankhauser and Moore (1941) on androgenetic eggs of *Triturus viridescens*. There have been a number of cytological studies on parthenogenesis in eggs of frogs (review of literature, Parmenter, 1933), but these have been concerned more with the rôle of the nucleus in early development of the egg (Dalcq, 1932) or with the origin of diploid and higher numbers of chromosomes (Parmenter, 1933, 1940) in cells of eggs and embryos rather than some of the factors underlying a failure of development beyond certain stages.

In view of the scarcity of studies on the cytology of early stages of androgenetic development in salamanders, the present study seemed to be indicated. It is a survey of the microscopical evidences of the causes of cessation of development in androgenetic eggs of *Triturus viridescens*. A preliminary cytological examination (Kaylor, 1939) showed that an irregular distribution of chromosomes had taken place in these eggs, as in the merogonic eggs of Fankhauser, and was probably responsible for the arrested development, since in this type of experiment no injury to the existing organization of the egg is possible.

Material and Methods

Material

During the course of experiments on androgenesis in *Triturus viridescens* (Kaylor, 1937, and later experiments not published) consider-

able material was preserved for future cytological studies. Of this material, 63 eggs which had ceased development during early cleavage, blastula or gastrula stages were selected for cytological examination. Fifty-nine of these eggs had actually completed their developmental possibilities; they were fixed either after they had remained in the same stage for 12 hours or more or at the onset of cytolysis as indicated by a beginning discoloration of some of the cells. Four of the eggs were preserved because of broken yolk membranes.

Methods

Experimental.—The technique used in obtaining these androgenetic eggs has already been described in detail (Kaylor, 1937). It consists essentially of the removal of the second maturation spindle from the egg by puncturing the polar area containing the spindle with a fine glass needle and sucking a small amount of material into a capillary pipette. The egg then develops with only the male, haploid set of chromosomes.

Fixation, Sectioning, Staining.—All eggs were fixed in Bouin's fluid, cleared from 95 per cent alcohol through wintergreen oil, and imbedded in paraffin containing about 5 per cent bayberry wax. This fixative hardens the yolk, but satisfactory sections were obtained by soaking the imbedded eggs in water for 12 to 24 hours, after the first 10 or 12 sections were cut and mounted: the method used by Fankhauser and Moore (1941). After this soaking, a complete ribbon of perfect sections was obtained. The sections were cut at 15 μ, parallel to the animal-vegetal axis. The sections were stained in Harris' acid-haemalum for the nuclear stain, eosin as a counterstain for the yolk granules, and Light green for the spindle fibers. They were then cleared from 95 per cent alcohol through pure aniline oil and mounted in an aniline-balsam mixture. The use of aniline was necessary since the use of xylene after the staining and dehydration processes always cracked the sections.

Figures 1 and 9 were drawn at a magnification of 80 and reduced to one-half in reproduction.

Observations

Observations on the Living Eggs

To review briefly the former observations on the living androgenetic eggs of *Triturus viridescens* (Kaylor, 1937), it was found first of all that although the majority of the androgenetic eggs underwent irregular cleavage and died prior to gastrulation, this abnormal cleavage was not

entirely responsible for the early cessation of development, since approximately one-half of the normally segmenting eggs failed to develop beyond the gastrula stage. Secondly, there existed no correlation between the type of cleavage of an androgenetic egg and the number of spermatozoa present in the egg at the time of operation. It was obvious, then, that a detailed cytological study of the early development of androgenetic eggs might determine the causes of the early arrested development.

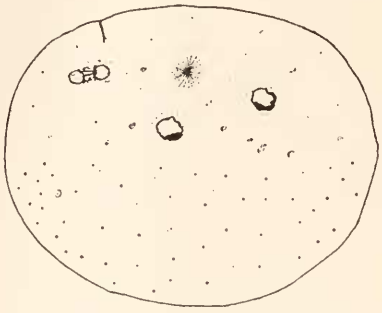


FIG. 1. Drawing of a median section of the egg 30.4e, sectioned parallel to the egg axis. All nuclei projected into this section from neighboring sections. Three degenerating sperm nuclei: two in prophase, one in telophase. One cytaster. Indication of a furrow.

Cytological Observations

The following stages of development of androgenetic eggs were examined in sections:

Stage of Development	Number of Eggs Examined
a. Irregular beginning cleavage	
1. Abortive cleavage	7
2. Early irregular cleavage	5
b. Early blastula	19
c. Late blastula	23
d. Gastrula	9
	Total 63

a. Irregular Beginning Cleavage Stages.—1. *Abortive cleavage.* Seven eggs were fixed 25 to 36 hours after operation, during which time only a few irregular, incomplete furrows had appeared on the egg surface. These furrows were still visible at the time the eggs were preserved. Surprisingly enough, in the sections there was no evidence of furrows in six of the seven eggs (Table I). One egg showed definite irregular furrowing, not connected with mitotic activity within the egg.

In each of these eggs there was evidence of early mitotic activity on the part of the sperm nuclei. The evidence is summarized in Table I. The cytological condition of each egg showed very little variation. The majority of sperm nuclei degenerated either before or after early mitotic activity. Cytasters were present in most of the eggs. Figure 1 illustrates the typical cytological condition encountered. In this particular egg, three or four sperm entrance marks were present on the egg surface at the time of operation. Three degenerating nuclei and

TABLE I

Summary of cytological conditions in abortive cleavage stages

Egg No.	No. Sperm Marks	Age	External Appearance	Cytological Condition
		hours		
26.1e	1	32	Irregular furrows at animal pole	No furrows, 2 nuclei degenerating in prophase, 1 spindle, no chromosomes
27.5e	3	36	Irregular furrows at animal pole	No furrows, 1 nucleus degenerating in prophase
28.6e	3	26	Irregular furrows at animal pole	No furrows, 3 nuclei degenerating in prophase
30.4e	3–4	26	One irregular furrow	Indication of furrows, 2 nuclei degenerating in prophase, 1 nucleus degenerating in telophase, 1 cytaster
30.5e	2	36	Irregular furrows at animal pole	No furrows, 1 cytaster, cytolysis
30.6e	4	25	Irregular furrows at animal pole	No furrows, 1 degenerating nucleus, 4 cytasters
101.1e	6	28	Irregular furrows at animal pole	No furrows, many degenerating nuclei

one cytaster were actually found in the egg; two of the nuclei were degenerating at prophase, and one at telophase.

2. *Early irregular cleavage.* Five eggs, fixed 24 to 26 hours after operation, were examined in this group. The cytological condition of each of these eggs is summarized in Table II.

From this table it is clear that the sperm nuclei in these eggs began to divide at the same or nearly the same time. One nucleus divided sooner than the others, but succeeded in forming only a few small, irregular cells. The "accessory" sperm nuclei either degenerated dur-

ing early mitosis, or continued to divide haphazardly. In any case, the presence of so many constellations in the egg does not lead to the formation of complete cleavage furrows.

Figures 2 and 3 illustrate the cytological condition of two of the most interesting eggs of this group. In the egg shown in Fig. 2, five

TABLE II

Summary of cytological conditions in early irregular cleavage stages

Egg No.	No. Sperm Marks	Age	External Appearance	Cytological Condition
		hours		
32.1e	6	26	Irregular cleavage	4–6 irregular "cells," no nuclei. In unsegmented region: 6 nuclei degenerating in prophase (monaster), 1 cytaster
34.3e	7+	26	Irregular cleavage	6–8 irregular cells, degenerating nuclei. In unsegmented region: 3 nuclei degenerating in prophase (monaster), 3 nuclei degenerating in metaphase (bipolar), 1 degenerating nucleus
61.1e	7	25	Irregular cleavage	6–8 irregular cells, degenerating nuclei. In unsegmented region: 3 nuclei degenerating in prophase (monastral), 1 nucleus degenerating in bipolar mitosis, 2 cytasters
63.1e	6	24	Irregular cleavage	4 irregular cells, mitosis in each. In unsegmented region: 5 nuclei degenerating in prophase (monastral), 9 cytasters, 1 triaster
64.2e	3	26	Irregular cleavage	many irregular cells, nuclei in majority degenerating. In unsegmented region: 4 small bipolar spindles, 7 degenerated nuclei, 3 large triastral mitoses with large number of chromosomes, 2 large tetrastral mitoses with large number of chromosomes, 10 cytasters

of the six spermatozoa entering the egg are degenerating after a beginning monastral mitosis. It is probable that the sixth sperm nucleus divided in a normal manner and was responsible for the formation of the few cells in the upper part of the egg. In four of these cells, a normal haploid mitosis is in progress. The nine cytasters scattered through the unsegmented part of the egg apparently have no connection with any of the sperm nuclei and for this reason probably originated

de novo in the cytoplasm, as they do in egg fragments of *Triton* (Fankhauser, 1934*a*). The large triaster is probably a fusion of three cytasters.

The cytological condition of the egg in Fig. 3 is much more complex. An inventory of the contents of this egg is given in Table II and in the explanation of Fig. 3. Since there were only three sperm entrance

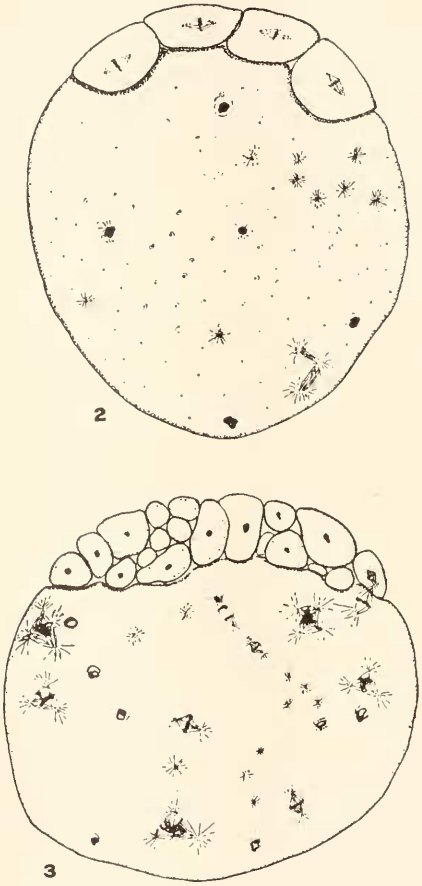
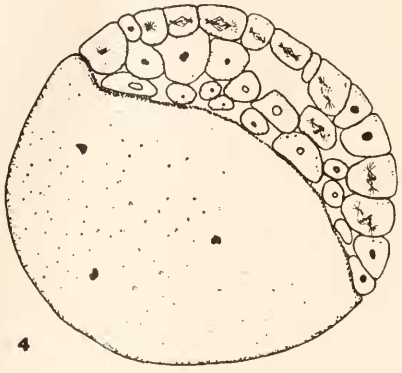


FIG. 2. Drawing of a median section of the egg 63.1e, sectioned parallel to the egg axis. All nuclei and cytasters projected into this section from neighboring sections. Irregular cells, mitosis in each. Five degenerating sperm nuclei, nine cytasters, one triaster in the unsegmented yolk region.

FIG. 3. Drawing of a median section of the egg 64.2e, sectioned parallel to the egg axis. All nuclei projected into this section from neighboring sections. Irregular cells, degenerating nuclei in most cells. In the unsegmented region: four small bipolar mitoses, seven degenerated nuclei, three large triastral mitoses and two large tetrastral mitoses with large numbers of chromosomes, ten cytasters.

marks on the living egg, it seems probable that only two sperm nuclei could have been responsible for the large number of irregular mitotic figures present, while the third sperm nucleus initiated the formation of the few small, irregular cells in the upper part of the egg. Several chromosome counts were made in the figures present in the yolk region.



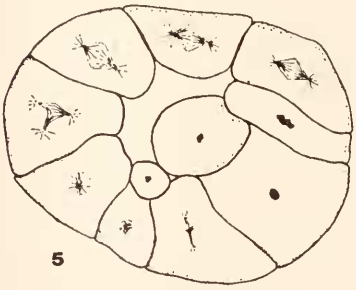


FIG. 4. Drawing of a median section of the egg 28.5e, sectioned parallel to the egg axis. Nuclei projected from neighboring sections into the cells and unsegmented region. Many cells non-nucleated, some with single asters, others with degenerating sperm nuclei in the yolk region.

FIG. 5. Drawing of a median section of the egg 30.7e, sectioned parallel to the egg axis. Nuclei projected from neighboring sections into the cells. Majority of cells non-nucleated. Fairly normal blastula.

In one normal anaphase figure, seventeen chromosomes were identified; eight at one pole and nine at the other (Fig. 10). In another anaphase, 18 chromosomes were identified, while in a nearby metaphase plate, 13 chromosomes could be counted. Several large, irregular triasters and tetrasters were in this yolk region. Large numbers of chromosomes were present in each of these figures.

b. Early Blastulae.—Nineteen eggs were fixed approximately 28 hours after operation, when they failed to develop beyond the mid-blastula stage. The most conspicuous features of the sections of these eggs were, first, that in 13 eggs a large area of the vegetative region was unsegmented. Only 6 eggs were completely segmented. Secondly, closer examination revealed a large number of abnormal mitotic figures

TABLE III

Chromosome numbers in early androgenetic blastulae

Egg No.	Total Number of Analyzable Mitotic Figures	Chromosome Numbers									
		9	10–11	11	11–12	12–14	14–16	16–18	18+	22	22+
26.9e	6				2		2				2
20.21e*	6				1	2		1			2
28.5e	2	1				1					
34.2e	25		2	3	5	6	2	2	4	1	
37Ae	11				2	1		1	1		6
52Ae	6				1	3		2			
53Ae*	6				1	1	2	2			
54.3e	12		1	3	8						
68Ae	2					2					
70.2e	11	3	3	3	2						
208.16e	8					4		2			2
00.E	18				8	3		3			4

* Preserved while still developing.

in the cells and unsegmented regions; in the 19 eggs a total of 312 mitoses of the following types were observed:

(*a*) Pluripolar mitoses.
(*b*) Monastral mitoses.
(*c*) Bipolar mitoses with degenerating chromosomes.
(*d*) Bipolar mitoses with no chromosomes.

Figure 4 is an example of an egg of the group of thirteen eggs with the undivided vegetal region. The cytology of this egg is given in detail since, although the egg does not possess all of the irregularities listed above, it is in general illustrative of the cytological condition of this group of eggs. A large sector of the roof of this blastula is composed of cells without nuclei; each cell contains a small bipolar spindle with no chromosomes. Other cells nearby contain only a single aster. In a few cells, bipolar mitoses are in progress, but in several of these figures chromosomes are showing signs of degeneration. Figure 11 il-

lustrates an anaphase spindle in one of these cells. Four chromosomes are lagging on the spindle and show definite abnormal swelling. The large unsegmented yolk region of this egg contains three nuclei which are degenerating in early prophase. The undivided yolk region of similar eggs, however, contained a larger number of abnormal figures

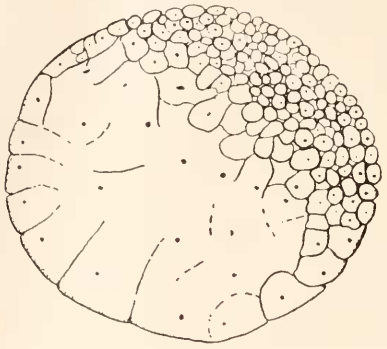


Fig. 6. Drawing of a median section of the egg 25.3e, sectioned parallel to the egg axis. Abnormal late blastula. Large areas of the yolk region unsegmented. No blastocoele.

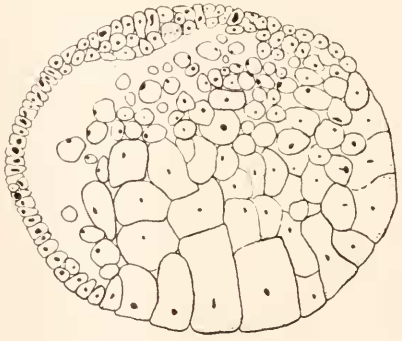


Fig. 7. Drawing of a median section of the egg 23.2e, sectioned parallel to the egg axis. Abnormal late blastula. Cells with pycnotic nuclei in segmentation cavity.

than are seen in this egg. Figures 12 and 13 show two of these mitoses.

The egg shown in Fig. 5 illustrates the typical condition in the group of six completely segmented eggs. The majority of cells contain bipolar spindles with no chromosomes; spindles similar to the one shown in Fig. 14. A few cells contain single cytasters. In other cells, the nuclei are degenerating. The small blastocoele has a few fragments

of cytoplasm containing no chromatin. The other five eggs did not have as many cells without nuclei.

Chromosome counts were possible in some of the cells of twelve of the nineteen eggs (Table III). In all but one of the eggs, the counts deviated from the haploid number (11 chromosomes) in the majority of cells. The one blastula which was haploid happened to have been fixed while still developing. It is doubtful that this egg could have reached an advanced stage of development because the cleavage was very irregular.

TABLE IV

Chromosome numbers in late androgenetic blastulae

Egg No.	Total Number of Analyzable Mitotic Figures	Chromosome Numbers									
		7–8	9–10	10–11	11	11–12	12–14	15–18	22	27+	30–33
23.2e	23	12	2				3	6			
25.3e	17	1		1	1	2	3	8	1		
25.9e	22	2	2	4	2	3	5	4			
26.5e	40	5	1	4	14	10		3	1	2	
26.6e*	40			14	15	8				2	1
27.1e*	26			3	5	5	9	4			
36.1e	19	1		4	6	6	1	1			
61.5e	11	1		3	5			2			
64.1e	11	2	7	2							
86.2e	9					1	2	4	1	1	
A.A.e	11			1	1		2	7			
3.Ae	15	1	1	4	2		2	4	1		
56.Ae	13	2	3	3	3			2			
60.Ae	12	1	1	4	1	1	2	2			

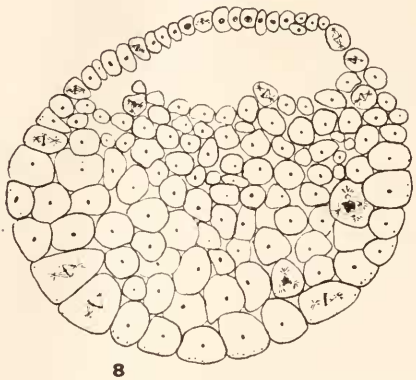
* Preserved while still developing.

c. Late Blastulae.—Of the 23 eggs fixed in the late blastula stage, only two were fixed while still developing. The following description will cover first of all the 21 eggs which had ceased development.

Although in external appearance each of these eggs resembled a normal blastula, the sections showed that all eggs were abnormal. Eighteen eggs were incompletely segmented in certain areas of the vegetal region. Only three eggs were completely segmented. All of the irregularities of mitosis observed in the earlier cleavage stages could be identified in the cells of these blastulae.

Since it would be impossible to describe the cytology of each of these eggs, the blastula shown in Fig. 6 was selected as representative of the group of 18 incompletely divided eggs. No blastocoele is present in the

egg. The upper half of the egg is composed of regularly segmented cells, while in the lower half the boundaries of many of the cells are incomplete. In sixty or more cells, the nuclear conditions were abnormal. The nuclei in the majority of these were degenerating, and in



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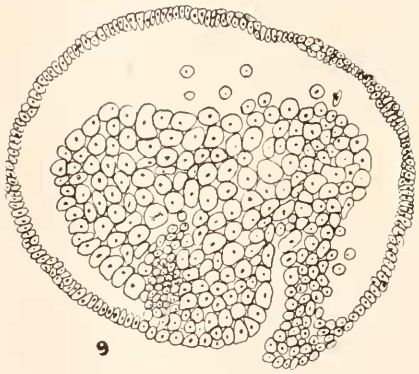

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Fig. 8. Drawing of a median section of the egg 26.6e which was preserved while still developing. Sectioned parallel to the egg axis. Fairly normal late blastula. Irregular mitoses beginning. Tetrastral mitosis in cell of vegetal region at right of drawing, one triastral mitosis in nearby cell.

Fig. 9. Drawing of a median section of the egg 37.3e, sectioned parallel to the egg axis. Abortive gastrula. Incomplete invagination of the yolk. Many cells with pycnotic nuclei in the blastocoele.

other cells, mitoses, still in progress, were frequently of a monastral type. Chromosome counts in 17 cells varied from 7 to 22 in number, indicating that irregular distributions of the chromosomes had occurred earlier in the cleavage history. Several mitotic figures in this egg showed stages of chromosome elimination. Figure 15 illustrates a

metaphase figure in which all of the chromosomes have degenerated. In another cell (Fig. 16), the chromatin is completely removed from the spindle. Other mitoses were observed in which the elimination of chromosomes was occurring more gradually; a few chromosomes at a time were being lost from the spindle. This is seen in Fig. 17. At least two and probably six chromosomes are not included in the metaphase group and will remain outside the nucleus in one of the two daughter cells. A telophase in a cell from another egg (Fig. 18) shows several degenerating chromosomes near the new cell membranes. These chromosomes will not be included in the daughter nuclei.

Each of the three completely segmented eggs possessed a segmentation cavity. Figure 7 illustrates one of these blastulae. About one-

TABLE V

Chromosome numbers in androgenetic gastrulae

Egg No.	Total Number of Analyzable Mitotic Figures	Chromosome Numbers										
		7–8	9–10	10	10–11	11	11–12	12	12–14	15–17	20–21	22+
26.11e	10										7	3
31.1e	14			14								
35.1e	12					8	4					
35.2e	18				6	10	2					
35.9e	6									4	2	
37.2e	25			2	1	7	3	9	1	2		
37.3e	21	1	2	2		7	4	2		3		
75.1e	10		2	2		2		4				
76.3e	9	2	2		1				1	3		

half of the roof of this blastula is composed of a double row of cells. The vegetal region still has abnormally large cells. A number of cells with pycnotic nuclei have separated from the yolk into the blastocoele. Although abnormal mitoses were not observed, an irregular distribution of chromosomes had occurred in earlier stages of development since chromosome numbers in 23 cells varied from 7 to 16 or 18.

Even though development was at a standstill in most of these eggs, mitoses were still frequent. The chromosomes of metaphase plates could be counted accurately in 14 eggs (Table IV). From Table IV it is clear that none of these blastulae were completely haploid.

Two late blastulae were preserved because of ruptured yolk membranes. One of these, Fig. 8, is most interesting because, unlike the majority of operated eggs, its cleavage had been undelayed and perfectly normal. There had been no suspicion, therefore, that the female nucleus

was actually out of the egg. In Fig. 8 it is seen that the egg was a fairly normal blastula. The first few chromosome counts were all haploid. Then the following mitoses were observed: (*a*) two normal bipolar figures with 22 chromosomes (the diploid number); (*b*) one triatral mitosis with 33 chromosomes (Fig. 19); and (*c*) a tetrastral figure with a large number of chromosomes, presumably the tetraploid number. One other cell (Fig. 20) contained a telophase figure with fragments of chromosomes at the center of the spindle. In view of the small number of cells with slightly irregular cytological conditions, this egg could probably have developed to a more advanced stage. The other egg possessed irregular chromosome numbers in the majority of cells. For this reason it probably would not have developed farther.

d. Gastrulae.—The last group of androgenetic eggs consisted of nine eggs fixed at the end of their development in the gastrula stage. In section, all of these eggs were found to be abortive gastrulae. The process of invagination of cells into the blastocoele was incomplete. In most of these eggs yolk cells with pycnotic nuclei were accumulating in the blastocoele (Fig. 9).

Although mitoses were not frequent in these gastrulae, a few chromosome counts were made in each egg (Table V). In all but two eggs, the majority of cells were not haploid. It is interesting to note that one gastrula had only 10 chromosomes in every cell clear enough for analysis. Apparently the lack of even one chromosome may be sufficient to disturb the processes of differentiation occurring for the first time at gastrulation.

The abnormal gastrulation of the two eggs which were completely haploid is not surprising since in later stages of development, as for example the formation of the neural plate, haploid embryos frequently have serious difficulties. This was observed in an earlier report (Kaylor, 1937), and in the experiments on the androgenetic development of frog embryos (Porter, 1939).

Discussion

The cytological conditions found in these eggs explain fully the high mortality rate during cleavage and gastrulation. In eggs fixed after irregular beginning cleavage, it was observed that either none of the sperm nuclei was sufficiently active to form cleavage furrows, or, quite the opposite, all of the sperm nuclei divided at the same or nearly the same time causing incomplete and irregular cleavage of the egg. The cytological conditions were somewhat the same in eggs which ceased de-

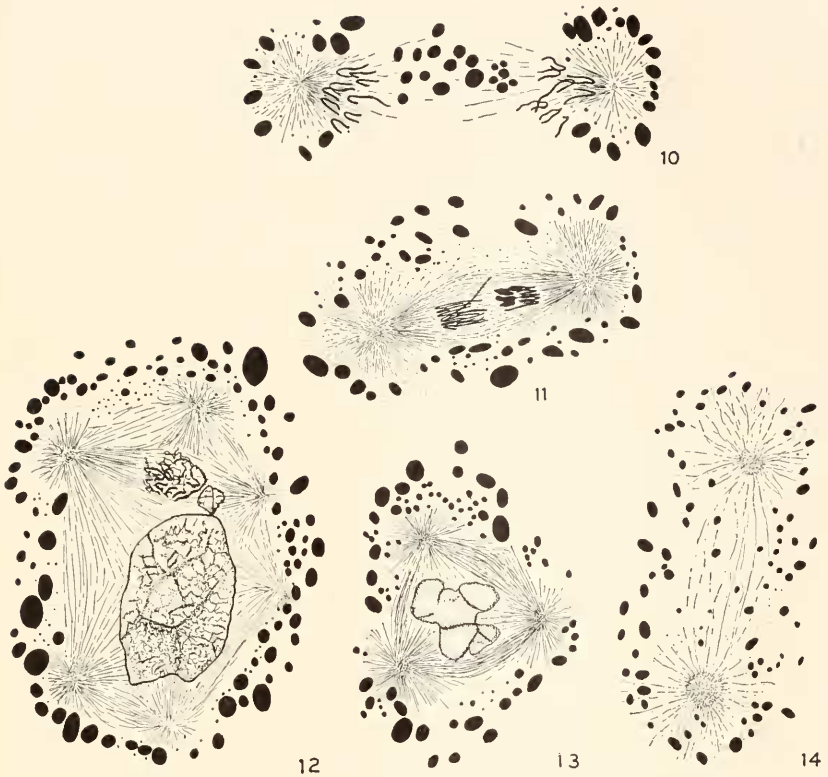

PLATE I

Explanation of Figures

Figures 10 to 14 were drawn at a magnification of 1200 and reduced to ca. 400 in reproduction.

Fig. 10. Anaphase figure in the yolk region of the egg in Text Fig. 3. Nine chromosomes at the upper pole and eight at the lower.

Fig. 11. Anaphase figure in a cell of the egg in Text Fig. 4. Four chromosomes, lagging on the spindle, show beginning degeneration.

Fig. 12. Pluripolar figure in the yolk region of the egg 37Ae. Apparently the fusion of several nuclei.

Fig. 13. Triastral figure in the yolk region of the egg 37Ae. Degenerating nucleus.

Fig. 14. Bipolar figure without chromatin in a cell of the egg 20.21e. The spindle shows a reduction in the number of spindle fibers.

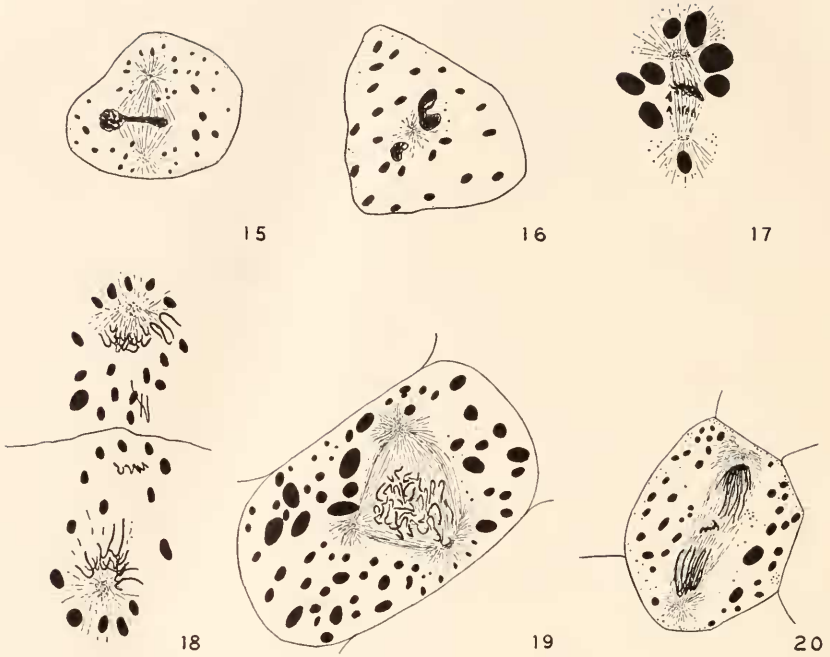

PLATE II

Explanation of Figures

Figures 15 to 20 drawn at a magnification of 1200 and reduced to ca. 600 in reproduction.

Figures 15 to 18, different stages in elimination of chromatin.

Fig. 15. Metaphase figure in a cell of the egg in Text Fig. 6. The chromosomes have degenerated into a pycnotic mass on the center of the spindle.

Fig. 16. Metaphase figure, polar view, in a cell of the egg in Text Fig. 6. The chromatin is completely removed from the spindle.

Fig. 17. Metaphase figure in a cell of the vegetal region of the egg in Text Fig. 6. Six chromosomes lagging on the spindle.

Fig. 18. Telophase mitosis in the egg AA.e. Several chromosomes lagging near the new cell membranes.

Fig. 19. Triastral figure in a cell of the egg in Text Fig. 8. Thirty-three chromosomes present.

Fig. 20. Anaphase figure in a cell of the egg in Text Fig. 8. Several chromosomes lagging on the spindle.

velopment during the early blastula stage. The majority of these eggs were incompletely segmented and contained abnormal nuclei in the cells and in the undivided areas, indicating the early irregular division of more than one sperm nucleus. The few completely segmented blastulae, although fairly normal in their cleavage, were, nevertheless, very irregular in their nuclear conditions. In these cases, the division of only one sperm nucleus probably initiated the almost normal cleavage, but even this early mitosis must have been extremely irregular.

The majority of eggs which had ceased development during the late blastula stage were incompletely segmented in certain areas of the egg. Only a few were normally formed blastulae. Chromosome counts in these eggs showed conclusively that irregular distribution of the male chromosomes had occurred earlier, and, indeed, was still going on in many cells at the time the eggs were preserved. It was of interest to note in the case of the normal androgenetic blastulae preserved while still developing, that one of these eggs possessed irregular chromosome numbers in the majority of mitoses analyzed. In the other egg, it was observed that irregular mitoses were just beginning. Cytological conditions such as these in normally developing androgenetic blastulae would be of importance in experiments involving the transplantation of haploid cells.

The nuclear conditions of the gastrulae were abnormal. About 80 per cent of the eggs ceasing development in this stage were not haploid. All of these gastrulae were abortive. Since it has been shown previously (Kaylor, 1937) that all androgenetic embryos which develop beyond the gastrula are haploid, it is apparent that the early gastrula is as far as an androgenetic egg can develop unless it possesses at least the haploid number of chromosomes in all of its cells.

These observations are in exact agreement with Fankhauser's (1934, *b*) conclusions from his excellent analysis of chromosome numbers and chromosome individuality in andro-merogonic *Triton* eggs. A complete discussion of the indispensability of a balanced set of chromosomes in early development is found in Fankhauser's papers.

These experiments on androgenesis have recently been extended to eggs of the Japanese newt, *Triturus pyrrhogaster* (Kaylor, 1940). In this species, a smaller percentage of the operated eggs die during blastula or gastrula stages. The more normal development of these eggs as compared with that in *Triturus viridescens* must be connected, then, with a more normal behavior of the sperm nuclei in early cleavage.

Summary

1. Androgenetic eggs of *Triturus viridescens* most frequently cease development in the following stages: *a*. Irregular beginning cleavage; *b*. Early blastula; *c*. Late blastula; *d*. Gastrula.

2. The causes of arrested development were investigated cytologically in eggs fixed in each of these stages.

3. Eggs of the first group were of two types, i.e., abortive cleavage, and early irregular cleavage in which a few cells were formed near the animal pole. In seven eggs of the first type, it was found that the sperm nuclei had degenerated either before or during early mitosis and cleavage furrows had disappeared. In five eggs of the second type, either all sperm nuclei had degenerated during early mitosis or one sperm nucleus divided more or less normally while "accessory" sperm nuclei either degenerated or divided irregularly in the unsegmented part of the egg.

4. In nineteen early blastulae, thirteen were incompletely segmented and six, although irregularly segmented, were fairly normal blastulae. Associated with these abnormalities in the thirteen eggs were the independent division of sperm nuclei in the yolk region without segmentation of the cytoplasm, and the presence of abnormal mitoses in the majority of cells. In the six almost normal mid-blastulae, the greater number of cells contained abnormal nuclei. Chromosome counts varied from 9 to 22 + in twelve of the nineteen eggs in which analyses could be made.

5. In twenty-three late blastulae sectioned, the same abnormalities as found in the earlier blastulae were observed. The majority of eggs were incompletely segmented and all of the eggs contained abnormal mitotic figures in some of the cells. Chromosome counts were made in fourteen eggs. None of these blastulae were completely haploid.

6. Nine gastrulae examined were abortive. No abnormal mitotic figures were found in these eggs, but in seven gastrulae the chromosome numbers varied above and below the haploid number, indicating that abnormal mitoses had occurred during earlier cleavage stages. Two gastrulae were haploid and it is assumed that these are examples of the abnormalities which many haploid embryos exhibit when differentiation of parts or of structures first takes place.

7. These observations confirm and extend those of Fankhauser and of Fankhauser and Moore. In order to develop beyond the gastrula stage, an androgenetic egg must be at least completely haploid.

BIBLIOGRAPHY

Dalcq, A., 1932. Contribution á l'analyse des fonctions nucléaires dans l'ontogenese de la grenouille. IV. Modifications de la formule chromosomiale. *Arch. de Biol.*, **43**: 343–366.

FANKHAUSER, G., 1934*a*. Cytological studies on egg fragments of the salamander Triton. IV. The cleavage of egg fragments without the egg nucleus. *Jour. Exper. Zoöl.*, **67**: 349–393.

FANKHAUSER, G., 1934*b*. Cytological studies on egg fragments of the salamander Triton. V. Chromosome number and chromosome individuality in the cleavage mitoses of merogonic fragments. *Jour. Exper. Zoöl.*, **68**: 1–57.

FANKHAUSER, G., AND CAROLINE MOORE, 1941. Cytological and experimental studies of polyspermy in the newt, Triturus viridescens. II. The behavior of the sperm nuclei in androgenetic eggs (in the absence of the egg nucleus). *Jour. Morph.*, **68**: 387–423.

KAYLOR, C. T., 1937. Experiments on androgenesis in the newt, Triturus viridescens. *Jour. Exper. Zoöl.*, **76**: 375–394.

KAYLOR, C. T., 1939. Cytological studies on androgenetic embryos of Triturus viridescens which have ceased development. *Biol. Bull.*, **77**: 334.

KAYLOR, C. T., 1940. Studies on experimental haploidy in salamander larvae. I. Experiments with eggs of the newt, Triturus pyrrhogaster. *Biol. Bull.*, **79**: 397–408.

PARMENTER, C. L., 1933. Haploid, diploid, triploid, and tetraploid chromosome numbers and their origin in parthenogenetically developed larvae and frogs of Rana pipiens and Rana palustris. *Jour. Exper. Zoöl.*, **66**: 409–453.

PARMENTER, C. L., 1940. Chromosome numbers in Rana fusca parthenogenetically developed from eggs with known polar body and cleavage histories. *Jour. Morph.*, **66**: 241–260.

PORTER, K. R., 1939. Androgenetic development of the egg of Rana pipiens. *Biol. Bull.*, **77**: 233–257.